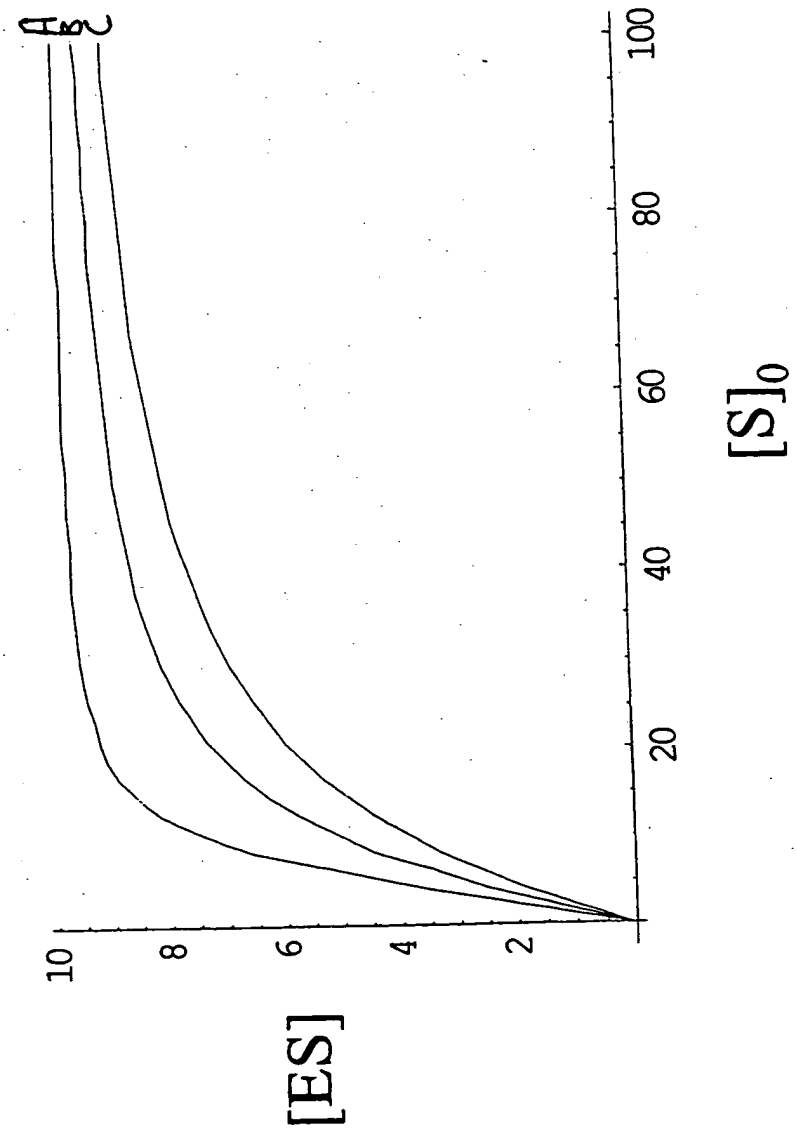
FIGURE 1



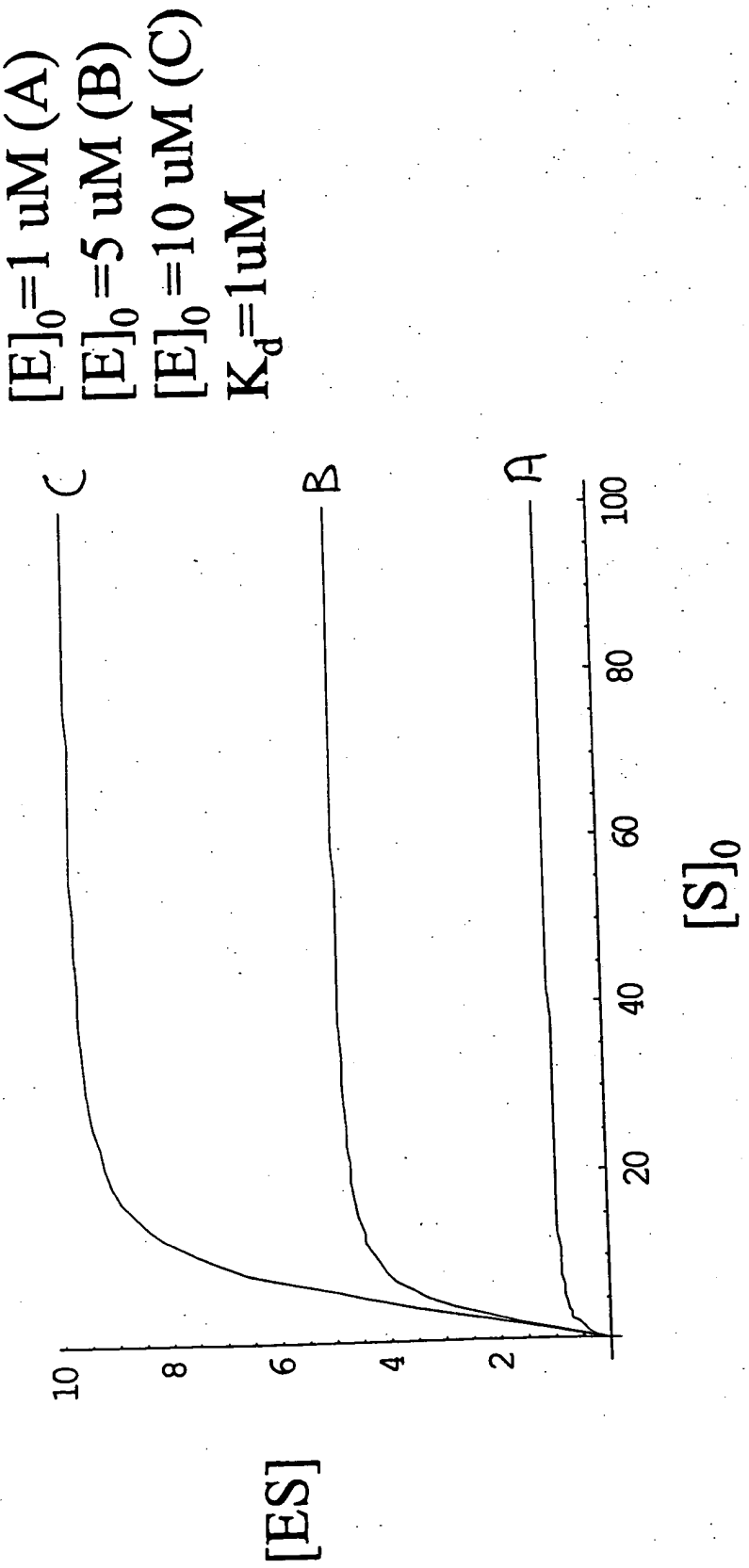
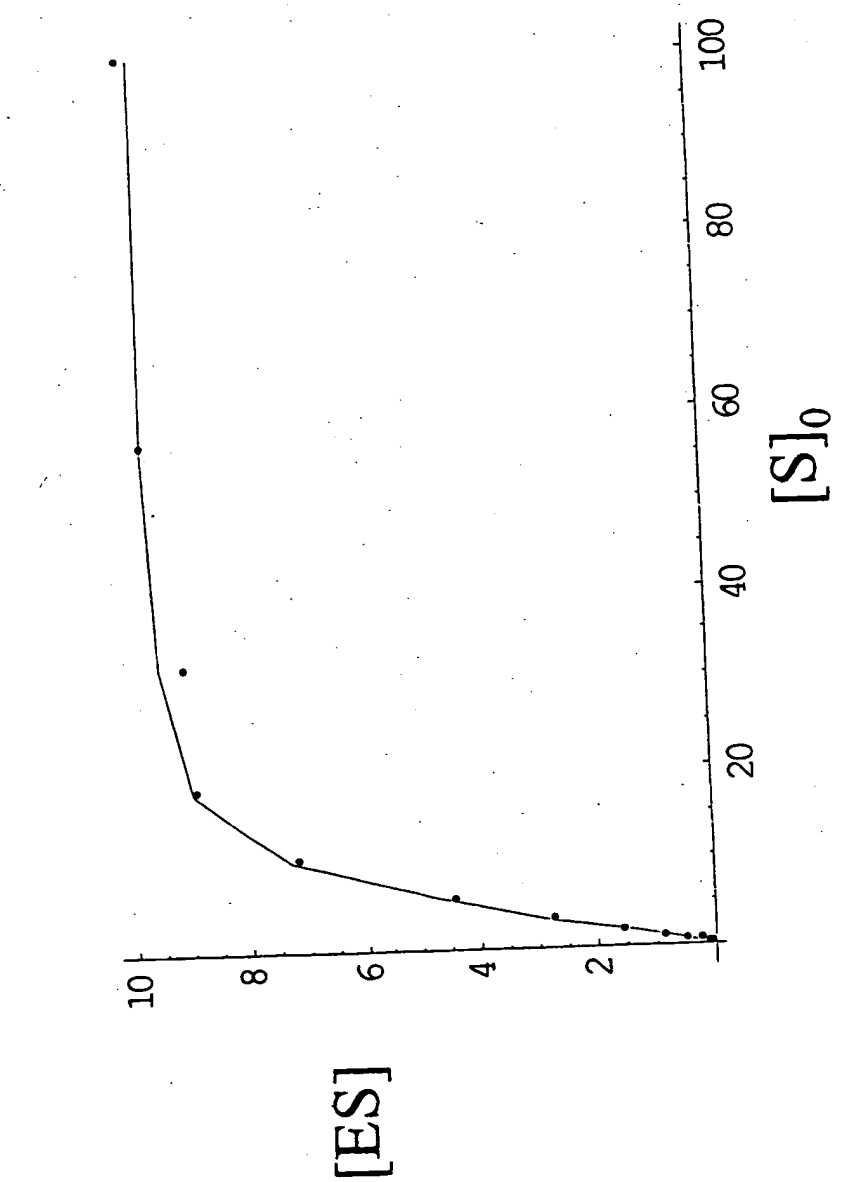


FIGURE 2

FIGURE 3


Actual values:
$[E]_0$=10.0 uM
K_d=1.0 uM
Best fit:
$[E]_0$=10.0 uM
K_d=1.1 uM
[ES]
10
8
6
4
2
20
40
60
80
100
$[S]_0$

$K_{d1} = 0.1\,\mu M$

$K_{d2} = 1.0$

$[S_1] = \text{variable}$

$[S_2]_0 = 10$

$[E]_0 = 1$

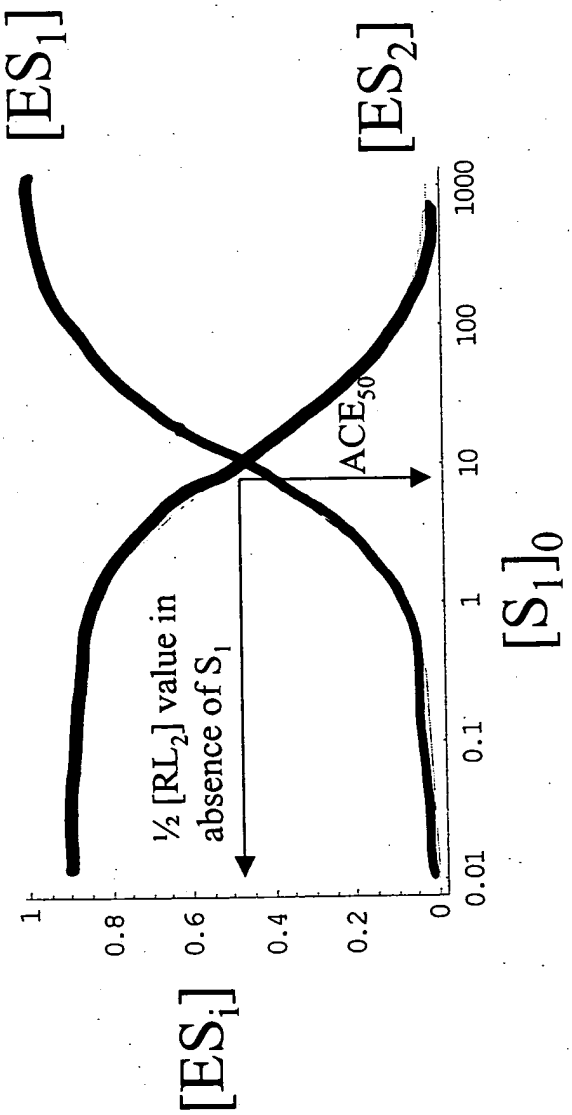


FIGURE 4

FIGURE 5A

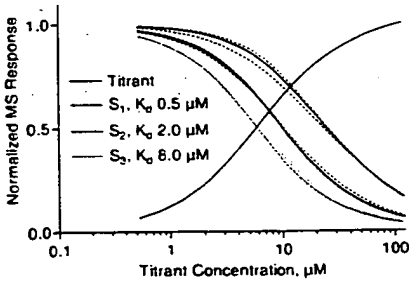


FIGURE 5B

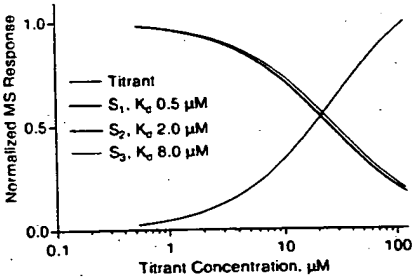
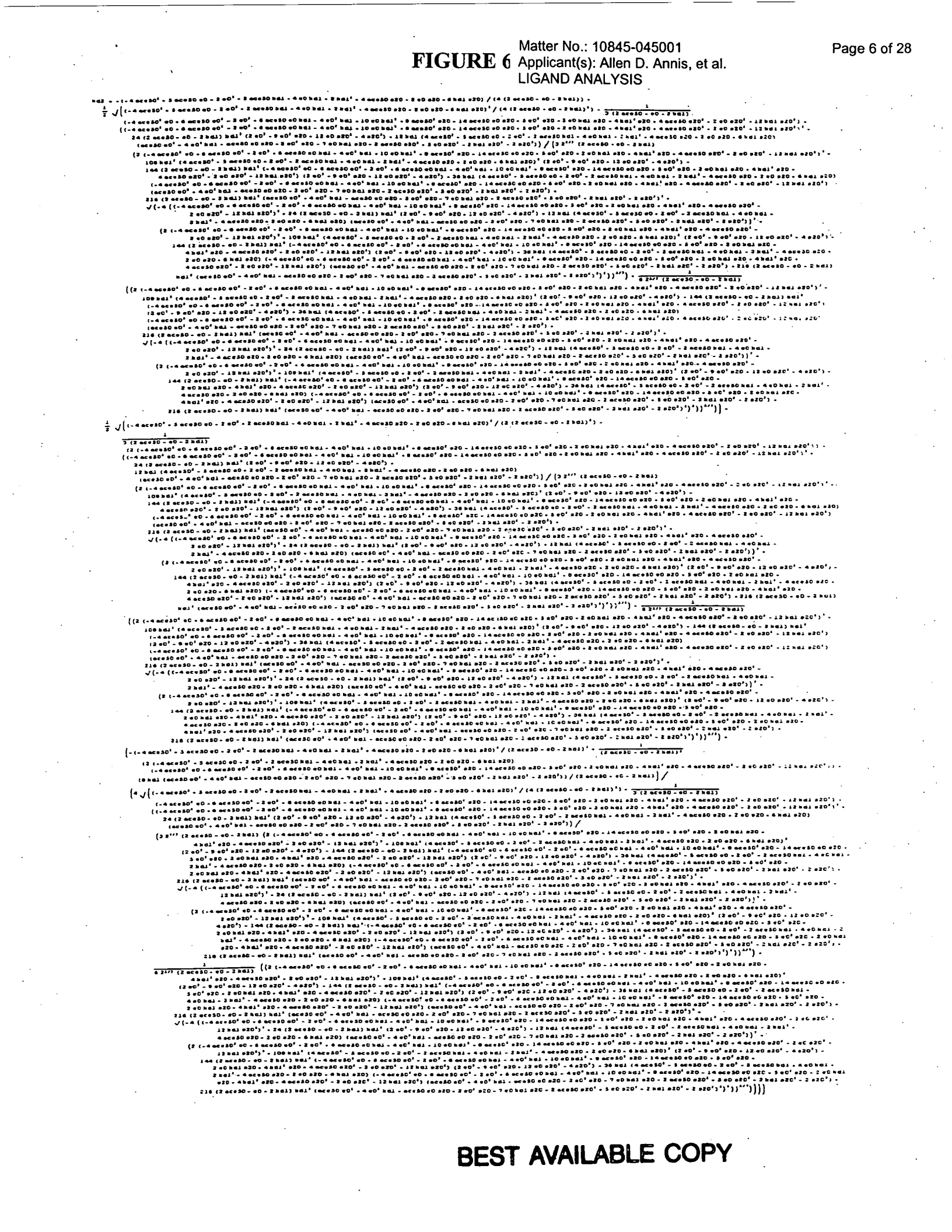


FIGURE 6



BEST AVAILABLE COPY

FIGURE 7

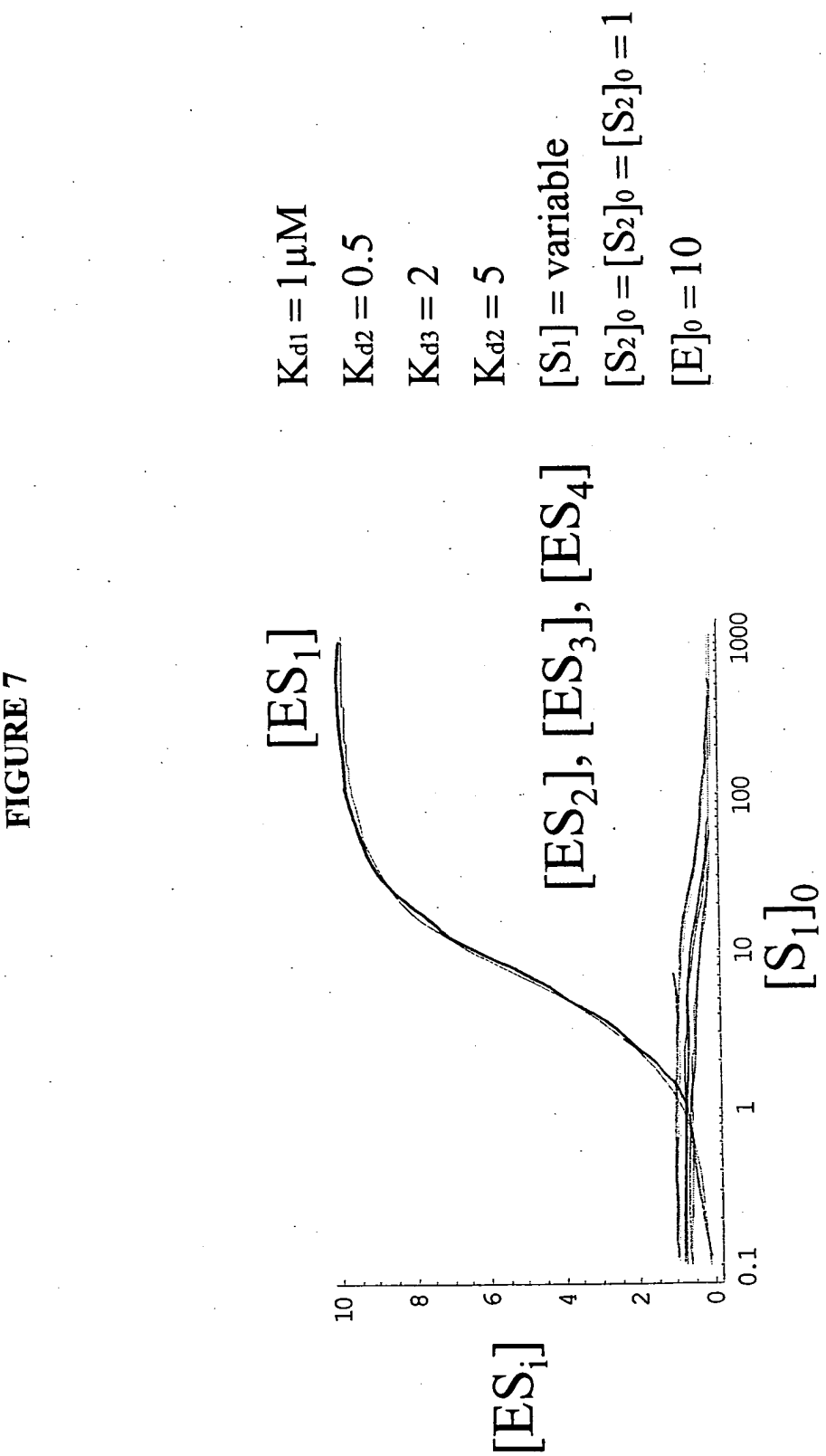
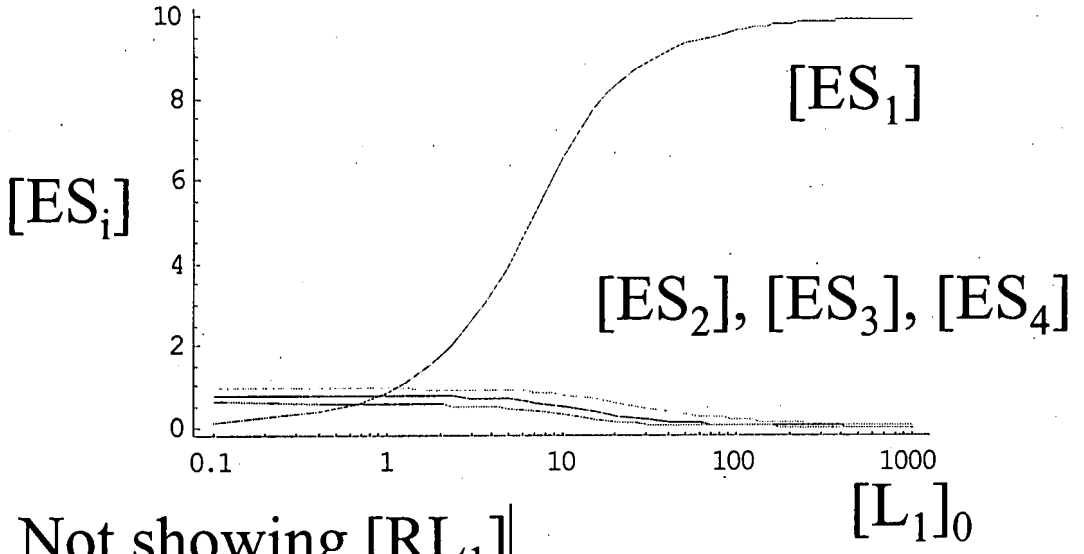

$K_{d1} = 1\mu M$
$K_{d2} = 0.5$
$K_{d3} = 2$
$K_{d2} = 5$
$[S_1]$ = variable
$[S_2]_0 = [S_2]_0 = [S_2]_0 = 1$
$[E]_0 = 10$
$[ES_1]$
$[ES_2]$, $[ES_3]$, $[ES_4]$
$[ES_i]$
$[S_1]_0$
10
8
6
4
2
0
0.1
1
10
100
1000

FIGURE 8



$K_{d1} = 1\,\mu M$

$K_{d2} = 0.5$

$K_{d3} = 2$

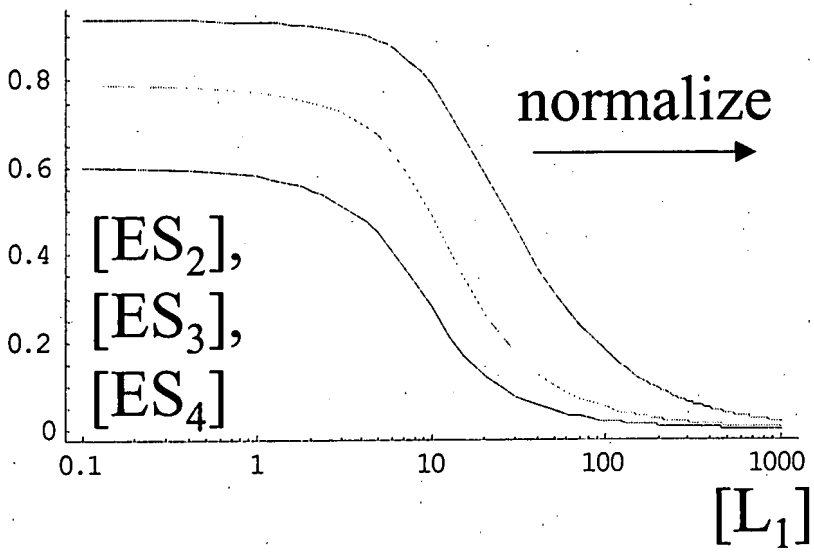
$K_{d4} = 5$

$[S_1] =$ variable

$[S_2]_0 = [S_3]_0 = [S_4]_0 = 1$

$[S]_0 = 10$

Not showing $[RL_1]$



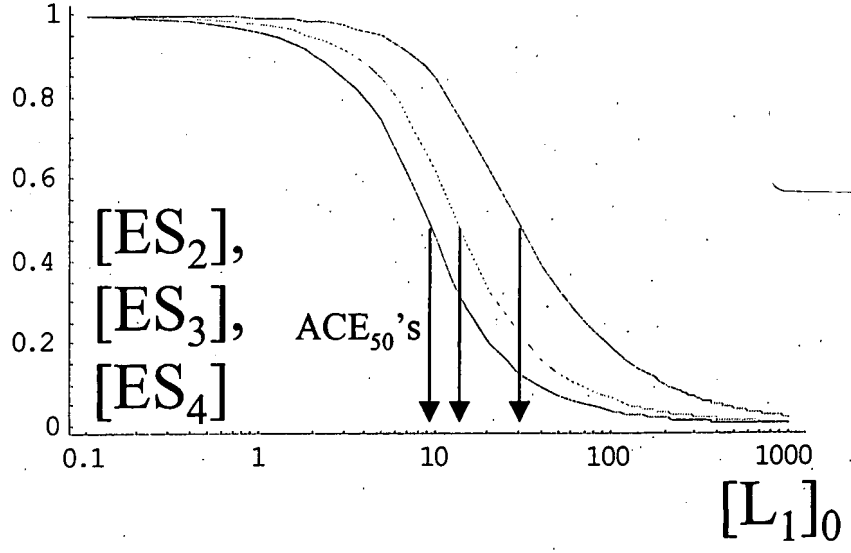


Figure-5 print.psd

FIGURE 10

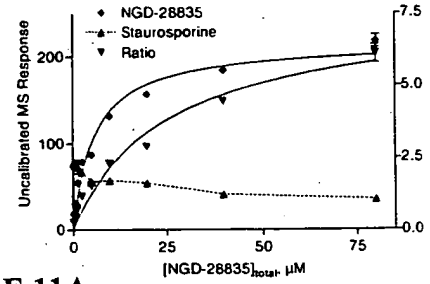


FIGURE 9

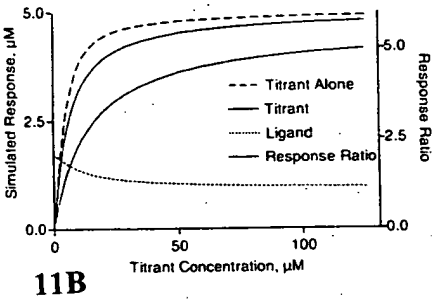
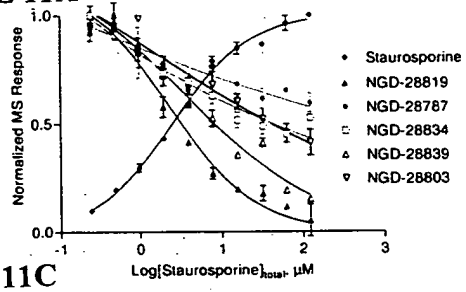
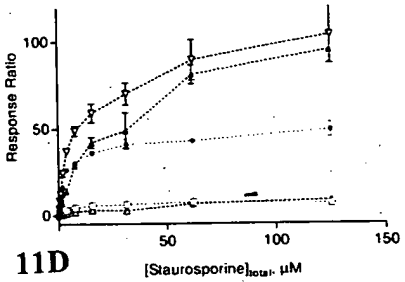


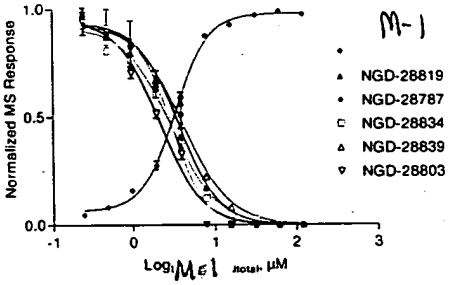
FIGURE 11A



11B



11C



11D

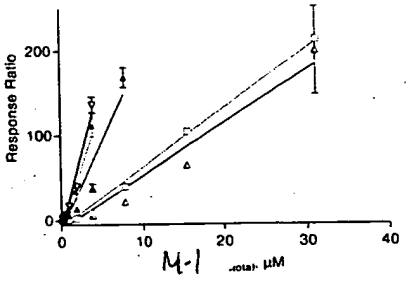


FIGURE 12

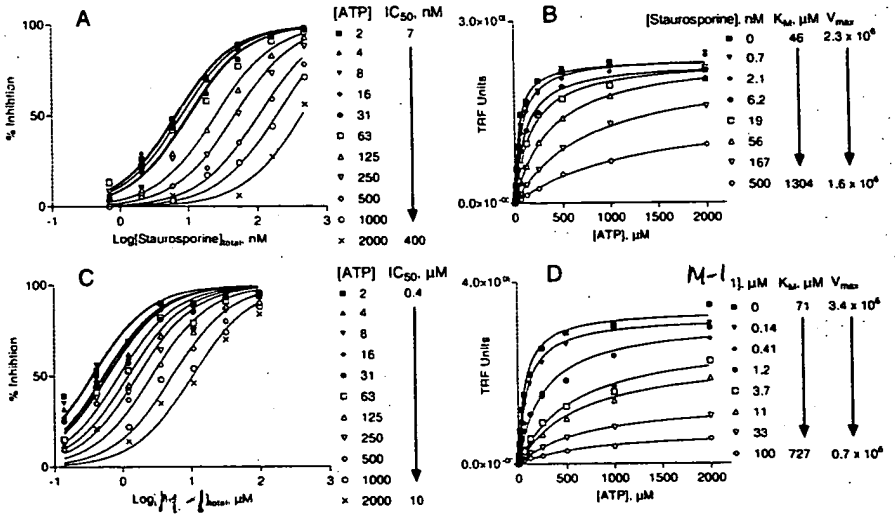

A
% Inhibition
Log[Staurosporine]total, nM
[ATP] IC50, nM
2 7
4
8
16
31
63
125
250
500
1000
2000 400
B
TRF Units
[ATP], µM
[Staurosporine], nM Km, µM Vmax
0 46 2.3 x 10^6
0.7
2.1
6.2
19
56
167
500 1304 1.6 x 10^6
C
% Inhibition
[ATP] IC50, µM
2 0.4
4
8
16
31
63
125
250
500
1000
2000 10
D
TRF Units
[ATP], µM
[M-I], µM Km, µM Vmax
0 71 3.4 x 10^6
0.14
0.41
1.2
3.7
11
33
100 727 0.7 x 10^6

FIGURE 13

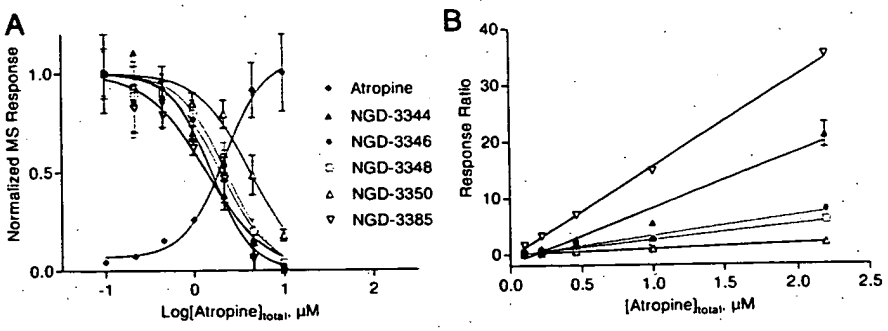

A
Normalized MS Response
1.0
0.5
0.0
-1
0
1
2
Log[Atropine]total, µM
Atropine
NGD-3344
NGD-3346
NGD-3348
NGD-3350
NGD-3385
B
Response Ratio
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
[Atropine]total, µM

FIGURE 14

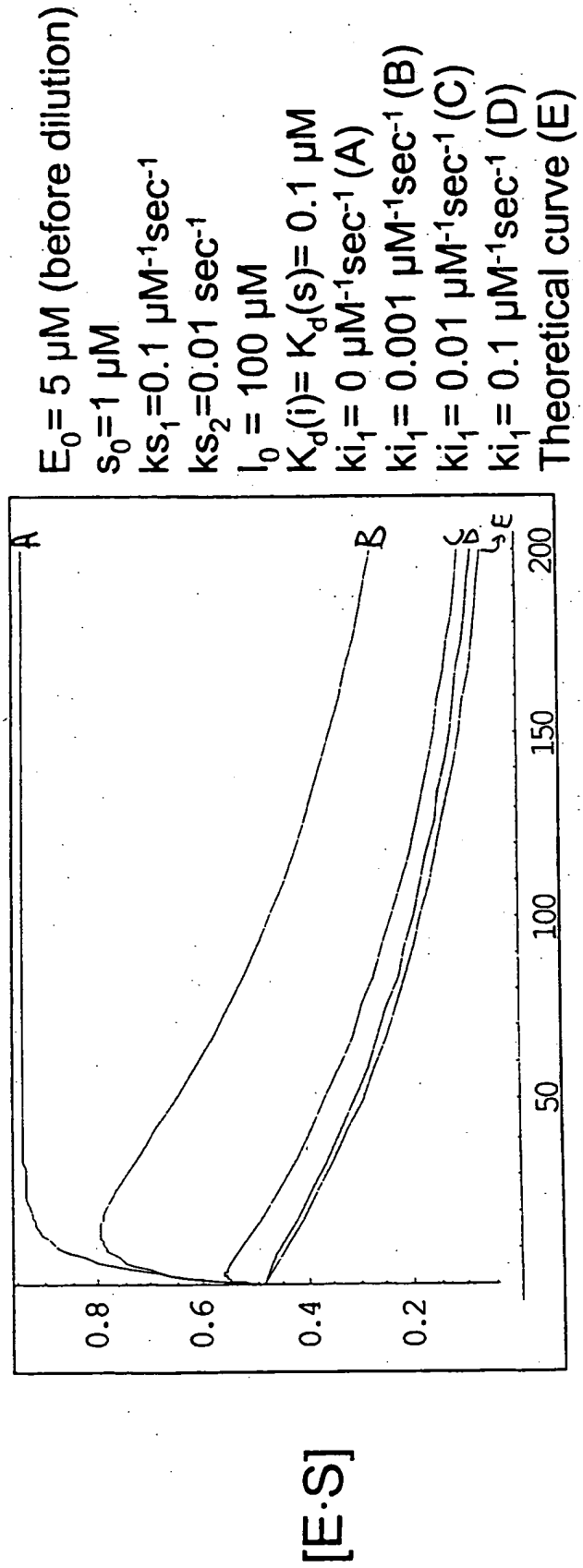

E_0= 5 µM (before dilution)
s_0=1 µM
ks_1=0.1 µM^{-1}sec^{-1}
ks_2=0.01 sec^{-1}
I_0 = 100 µM
$K_d(i)$= $K_d(s)$= 0.1 µM
ki_1= 0 µM^{-1}sec^{-1} (A)
ki_1= 0.001 µM^{-1}sec^{-1} (B)
ki_1= 0.01 µM^{-1}sec^{-1} (C)
ki_1= 0.1 µM^{-1}sec^{-1} (D)
Theoretical curve (E)
A
B
C
D
E
0.8
0.6
0.4
0.2
50
100
150
200
Time, sec
[E·S]

FIGURE 15

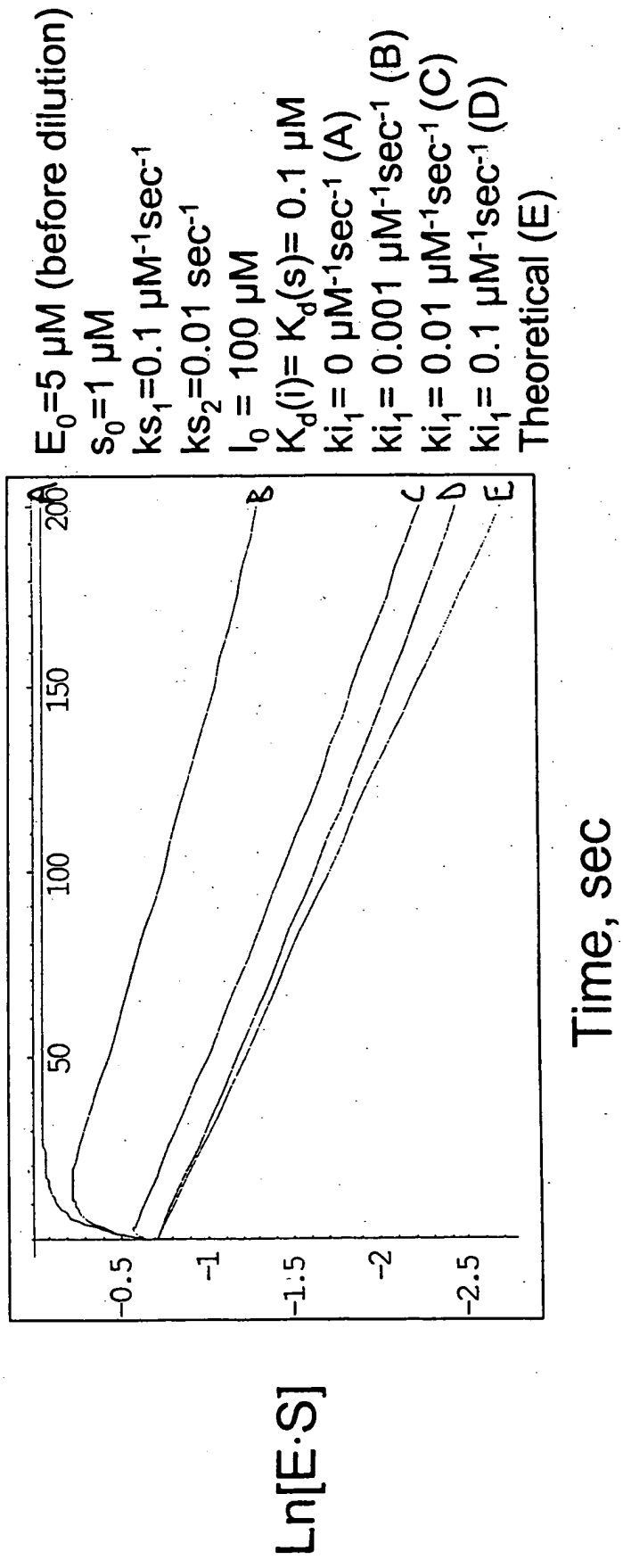
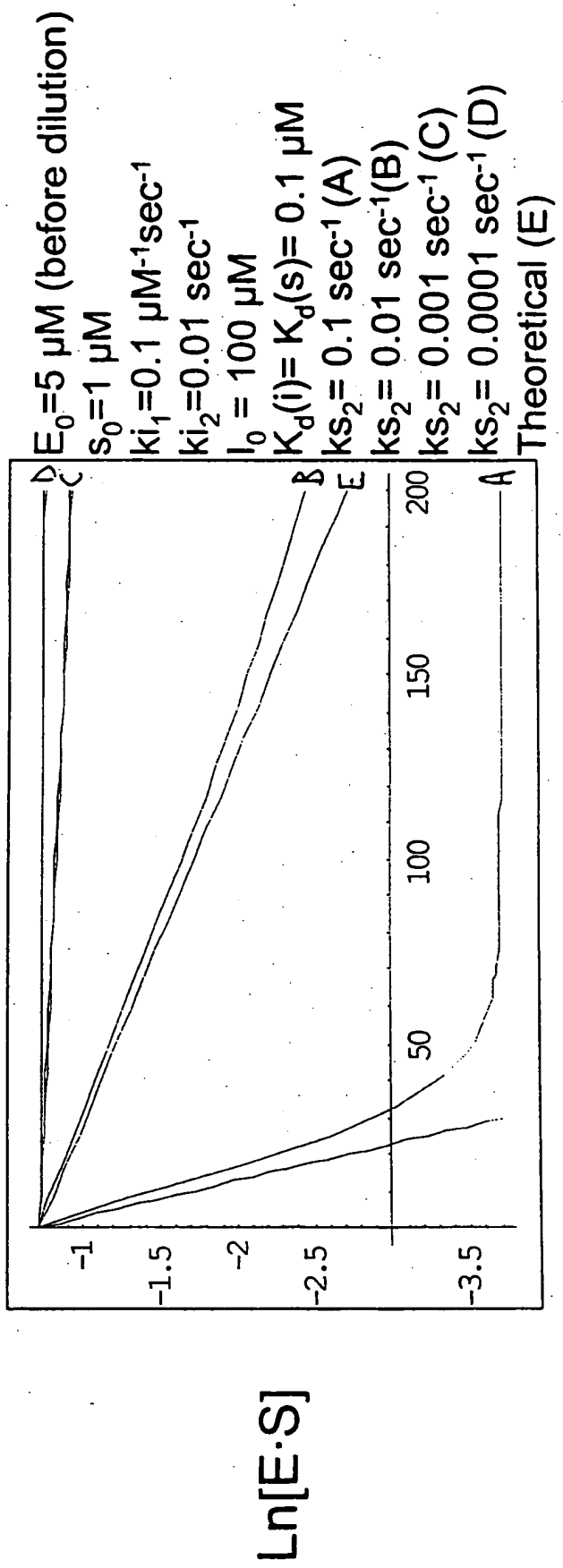

E_0=5 µM (before dilution)
s_0=1 µM
ks_1=0.1 µM^{-1}sec^{-1}
ks_2=0.01 sec^{-1}
I_0 = 100 µM
$K_d(i)$= $K_d(s)$= 0.1 µM
ki_1= 0 µM^{-1}sec^{-1} (A)
ki_1= 0.001 µM^{-1}sec^{-1} (B)
ki_1= 0.01 µM^{-1}sec^{-1} (C)
ki_1= 0.1 µM^{-1}sec^{-1} (D)
Theoretical (E)
A
B
C
D
E
200
150
100
50
-0.5
-1
-1.5
-2
-2.5
Ln[E·S]
Time, sec

FIGURE 16


E_0=5 µM (before dilution)
s_0=1 µM
ki_1=0.1 µM^{-1}sec^{-1}
ki_2=0.01 sec^{-1}
I_0 = 100 µM
$K_d(i) = K_d(s)$= 0.1 µM
ks_2= 0.1 sec^{-1} (A)
ks_2= 0.01 sec^{-1}(B)
ks_2= 0.001 sec^{-1} (C)
ks_2= 0.0001 sec^{-1} (D)
Theoretical (E)
D
C
B
E
A
-1
-1.5
-2
-2.5
-3.5
50
100
150
200
Ln[E·S]
Time, sec

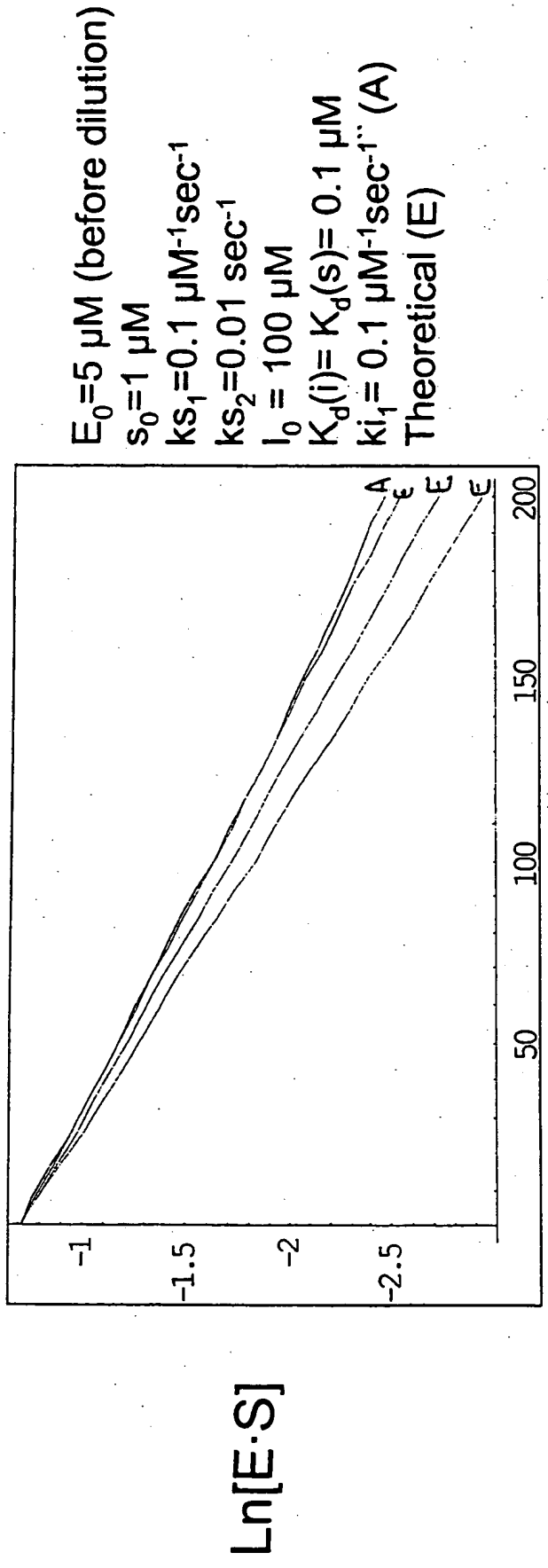
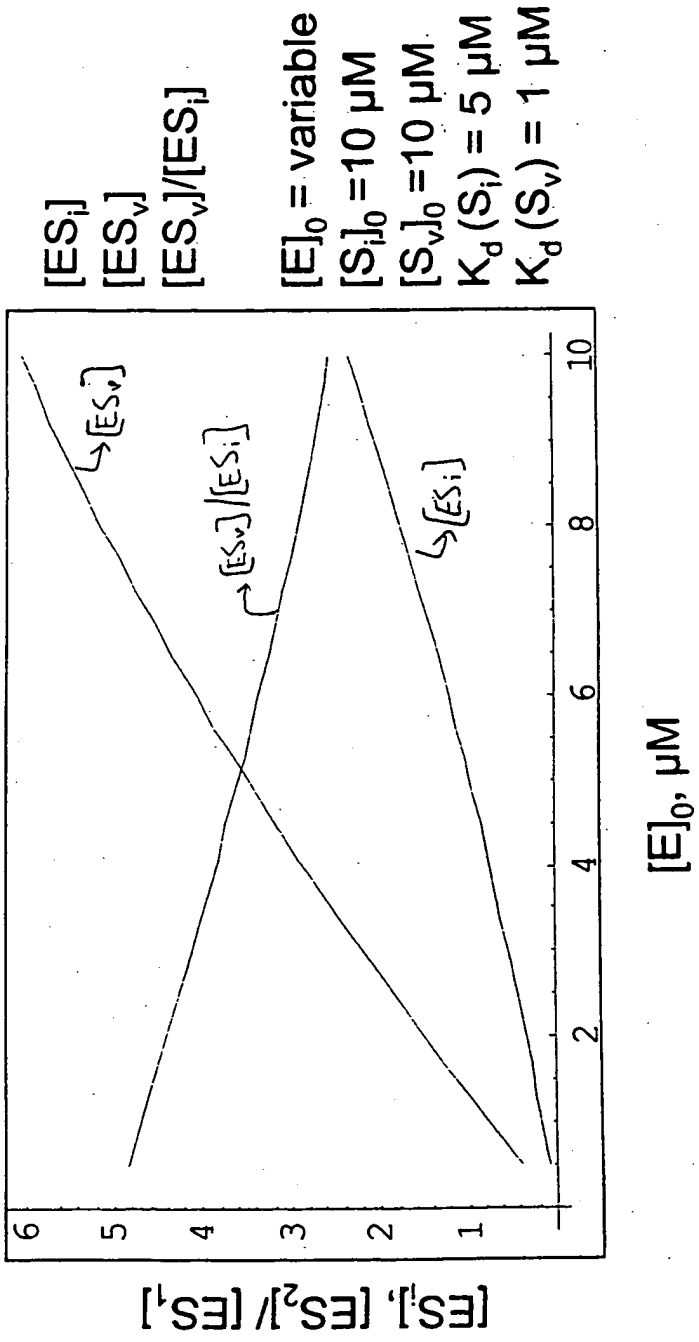

E_0=5 µM (before dilution)
s_0=1 µM
ks_1=0.1 µM^{-1}sec^{-1}
ks_2=0.01 sec^{-1}
I_0 = 100 µM
$K_d(i) = K_d(s)$= 0.1 µM
ki_1= 0.1 µM^{-1}sec^{-1} (A)
Theoretical (E)
A
E
E
E
-1
-1.5
-2
-2.5
50
100
150
200
Ln[E·S]
Time, sec

FIGURE 17

FIGURE 18


[ES$_i$]
[ES$_v$]
[ES$_v$]/[ES$_i$]
[E]$_0$ = variable
[S$_i$]$_0$ =10 µM
[S$_v$]$_0$ =10 µM
K_d (S$_i$) = 5 µM
K_d (S$_v$) = 1 µM
[ES$_v$]
[ES$_v$]/[ES$_i$]
[ES$_i$]
[ES$_i$], [ES$_2$]/ [ES$_1$]
[E]$_0$, µM
1
2
3
4
5
6
2
4
6
8
10

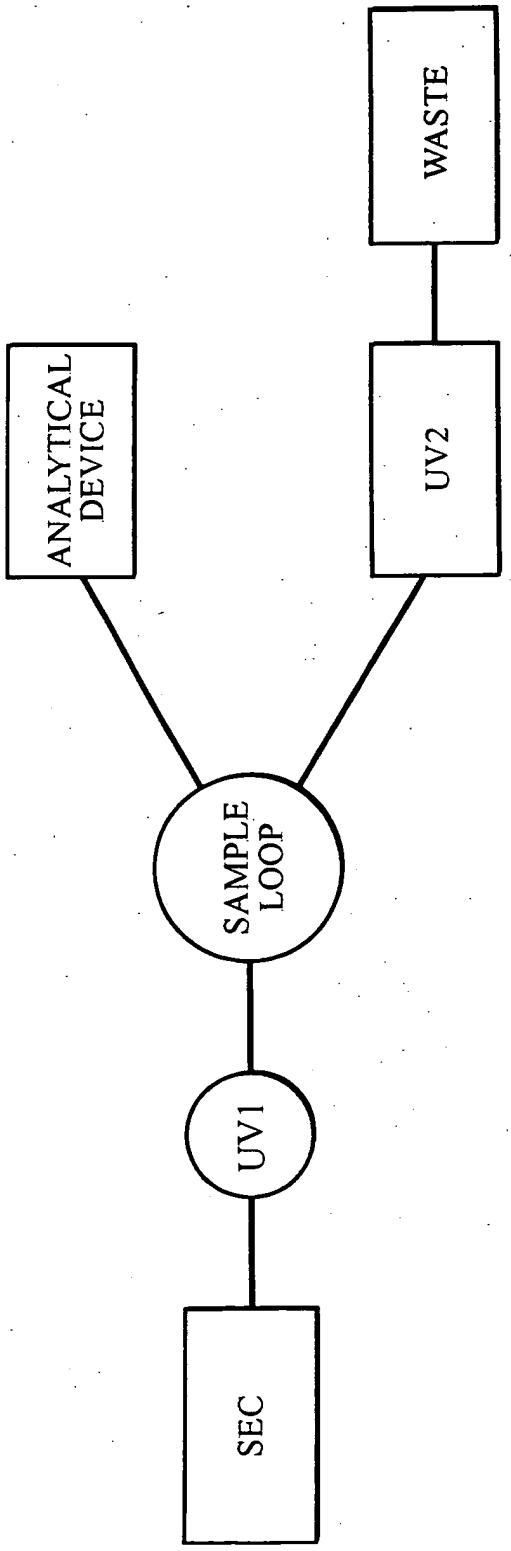


FIGURE 19

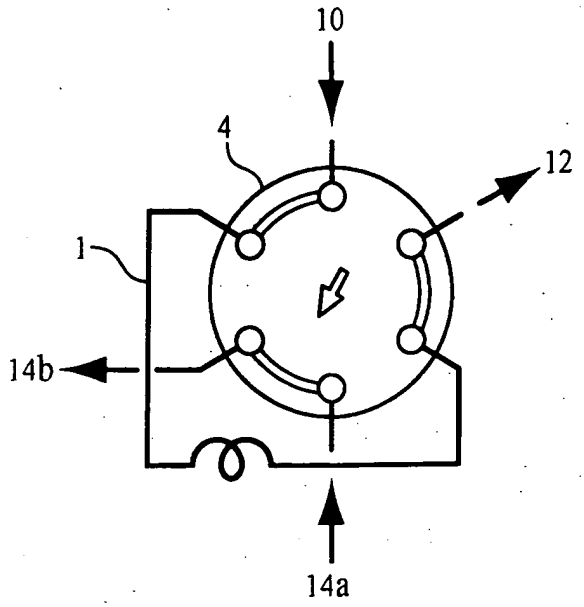


FIGURE 20A

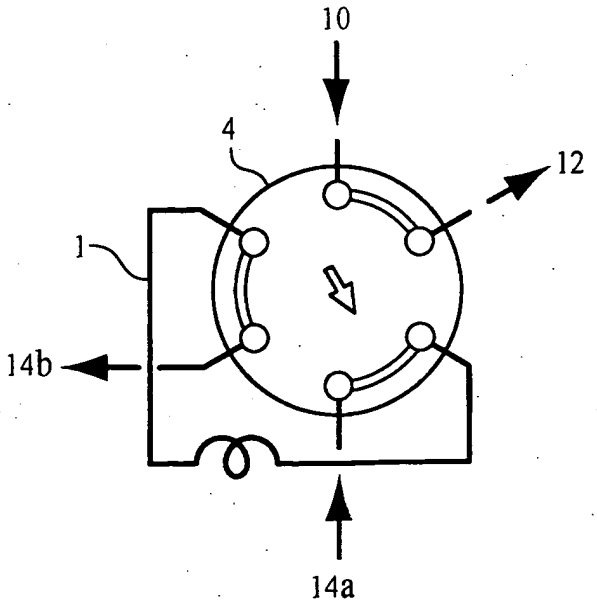


FIGURE 20B

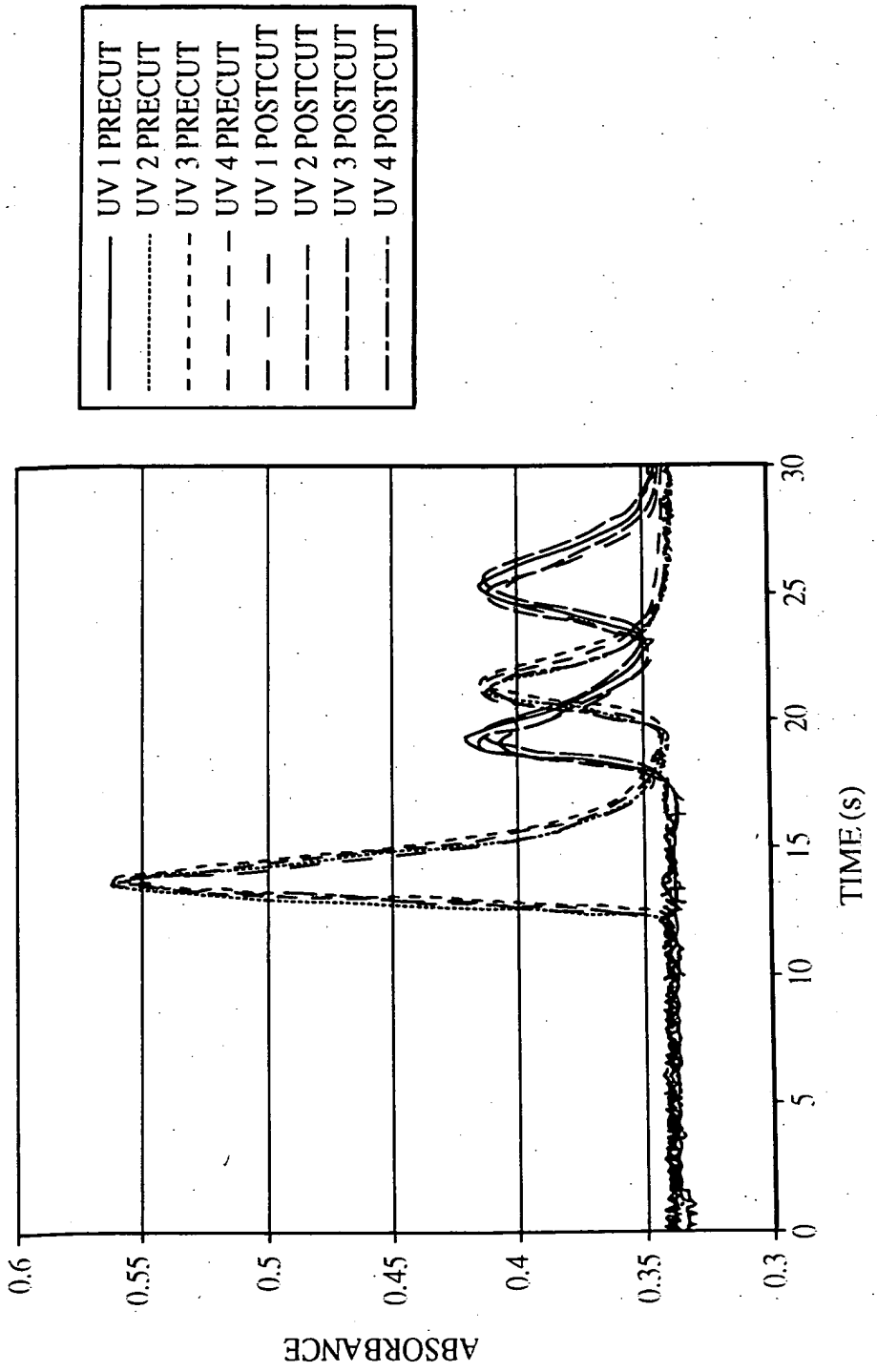

UV 1 PRECUT
UV 2 PRECUT
UV 3 PRECUT
UV 4 PRECUT
UV 1 POSTCUT
UV 2 POSTCUT
UV 3 POSTCUT
UV 4 POSTCUT
ABSORBANCE
0.6
0.55
0.5
0.45
0.4
0.35
0.3
TIME (s)
0
5
10
15
20
25
30

FIGURE 21

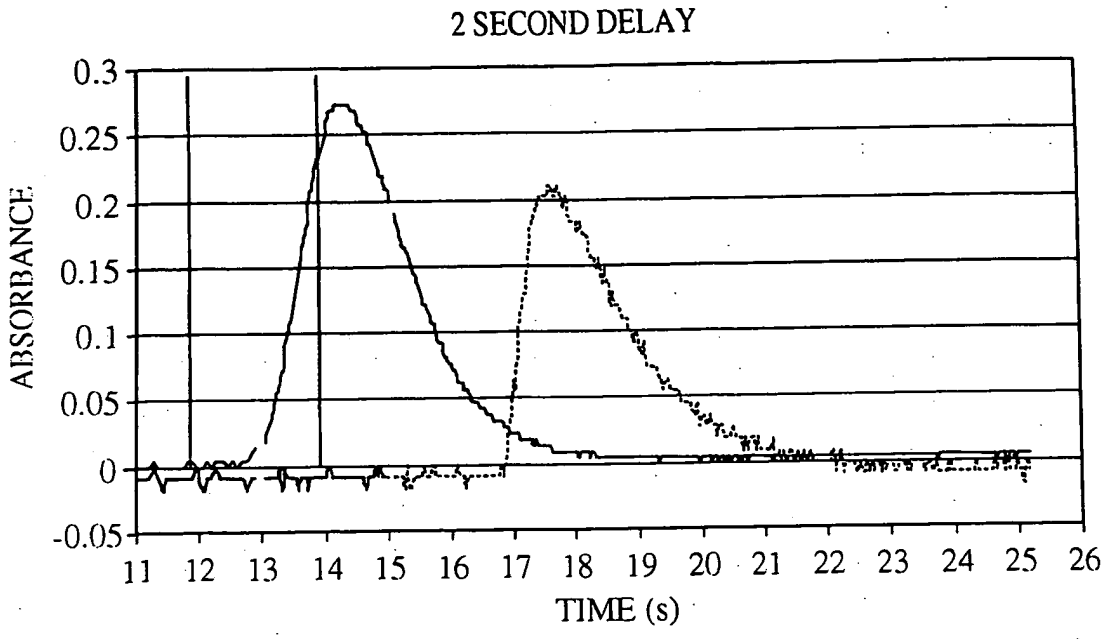


FIGURE 22A

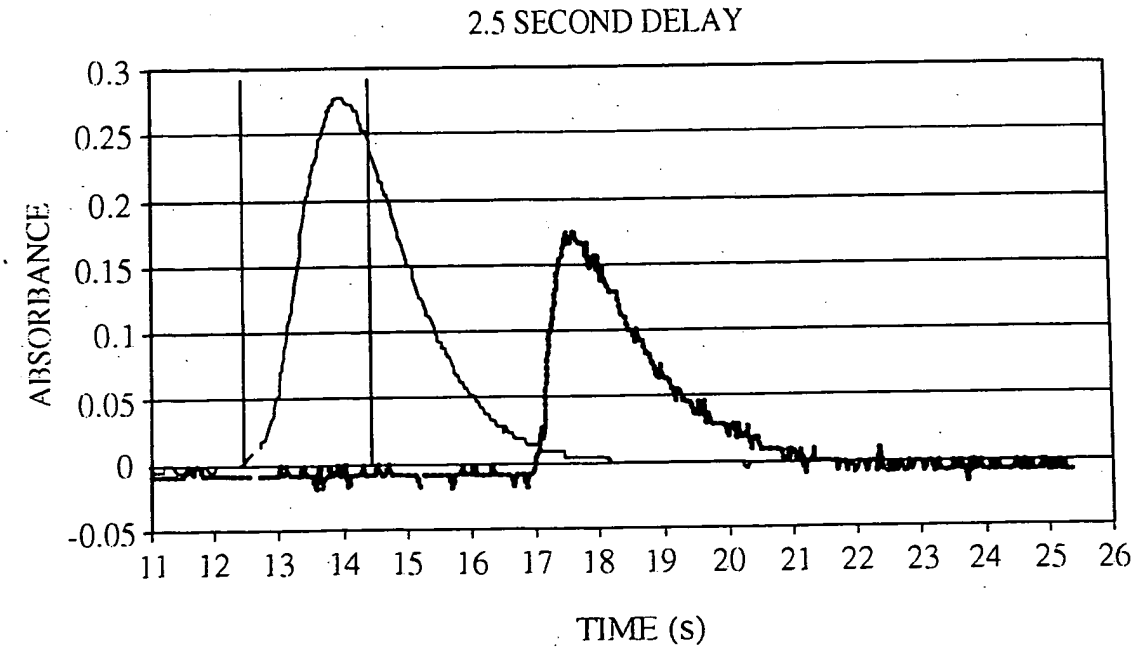


FIGURE 22B

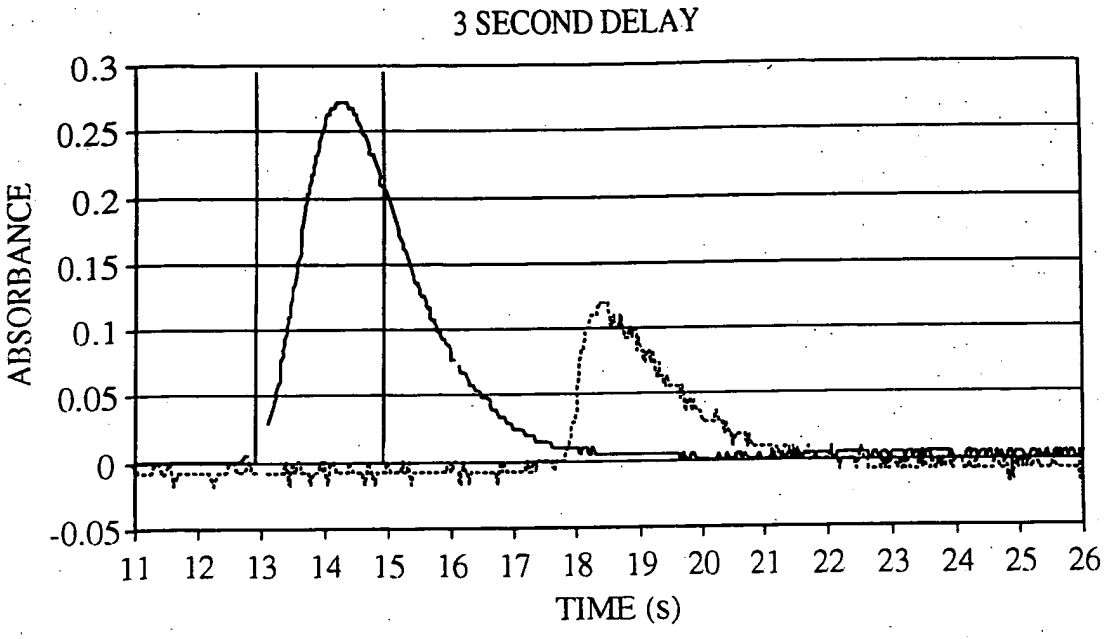


FIGURE 22C

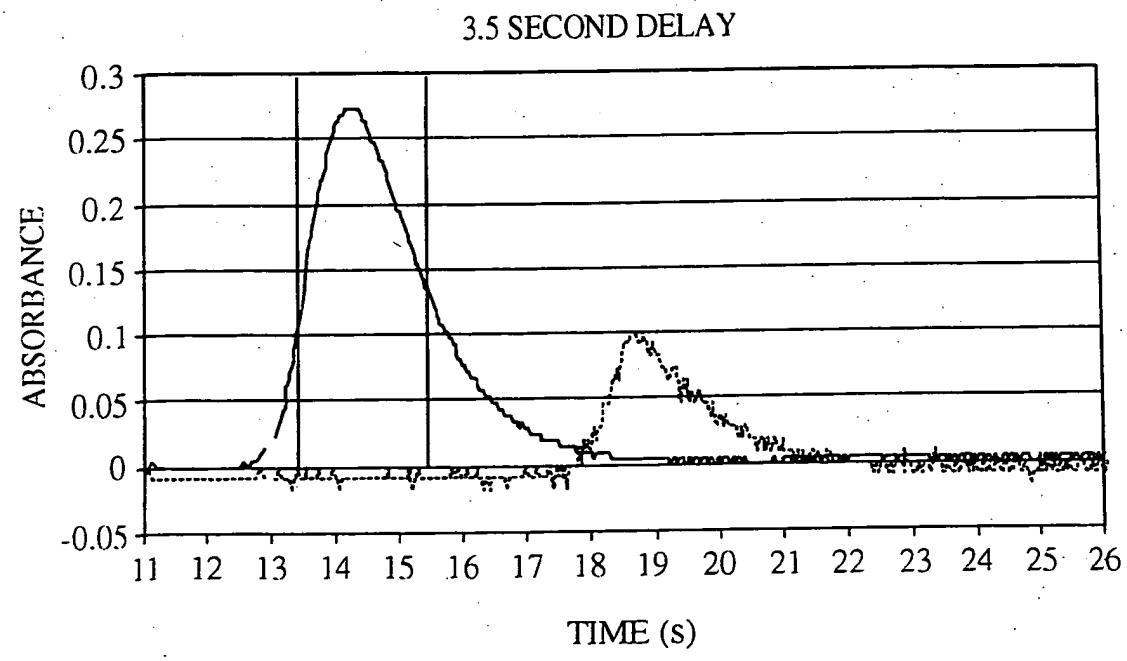


FIGURE 22D

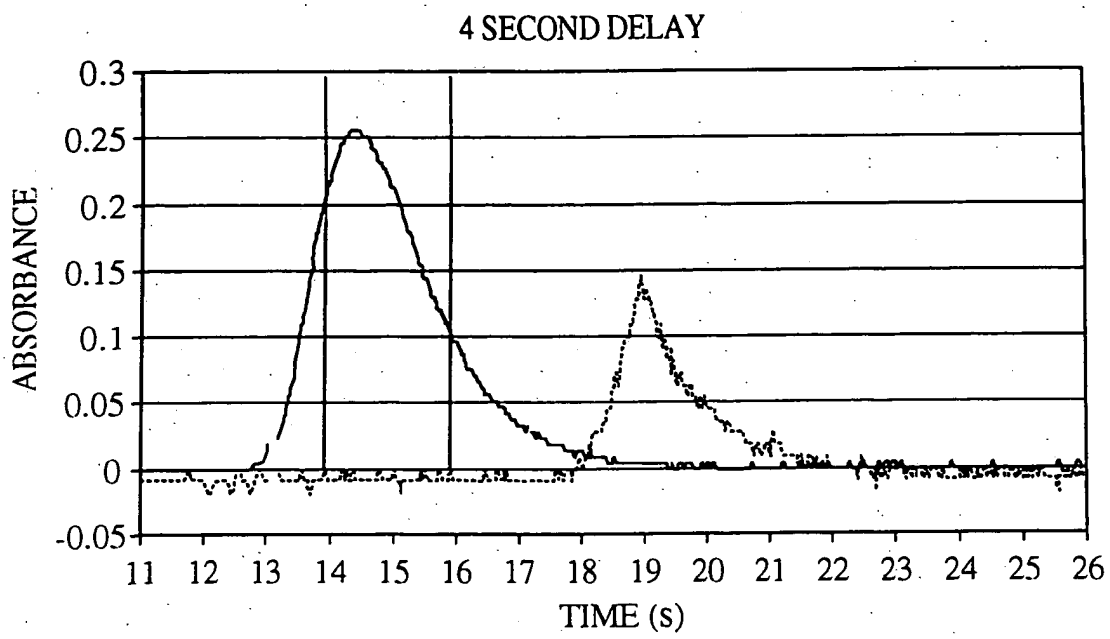


FIGURE 22E

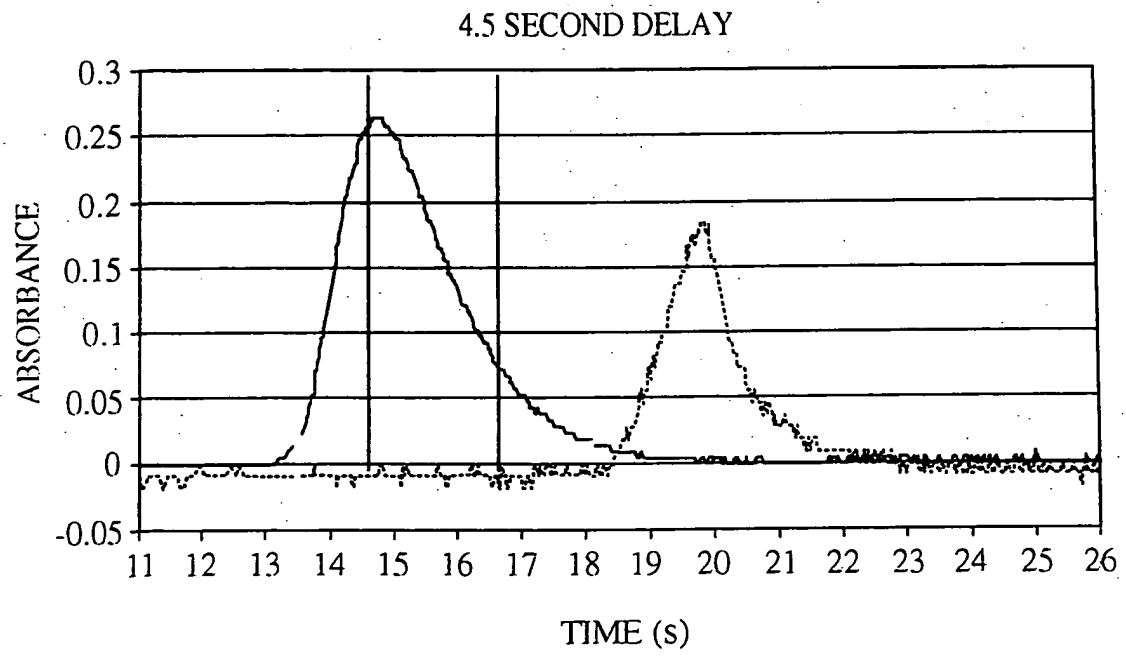


FIGURE 22F

FIGURE 23

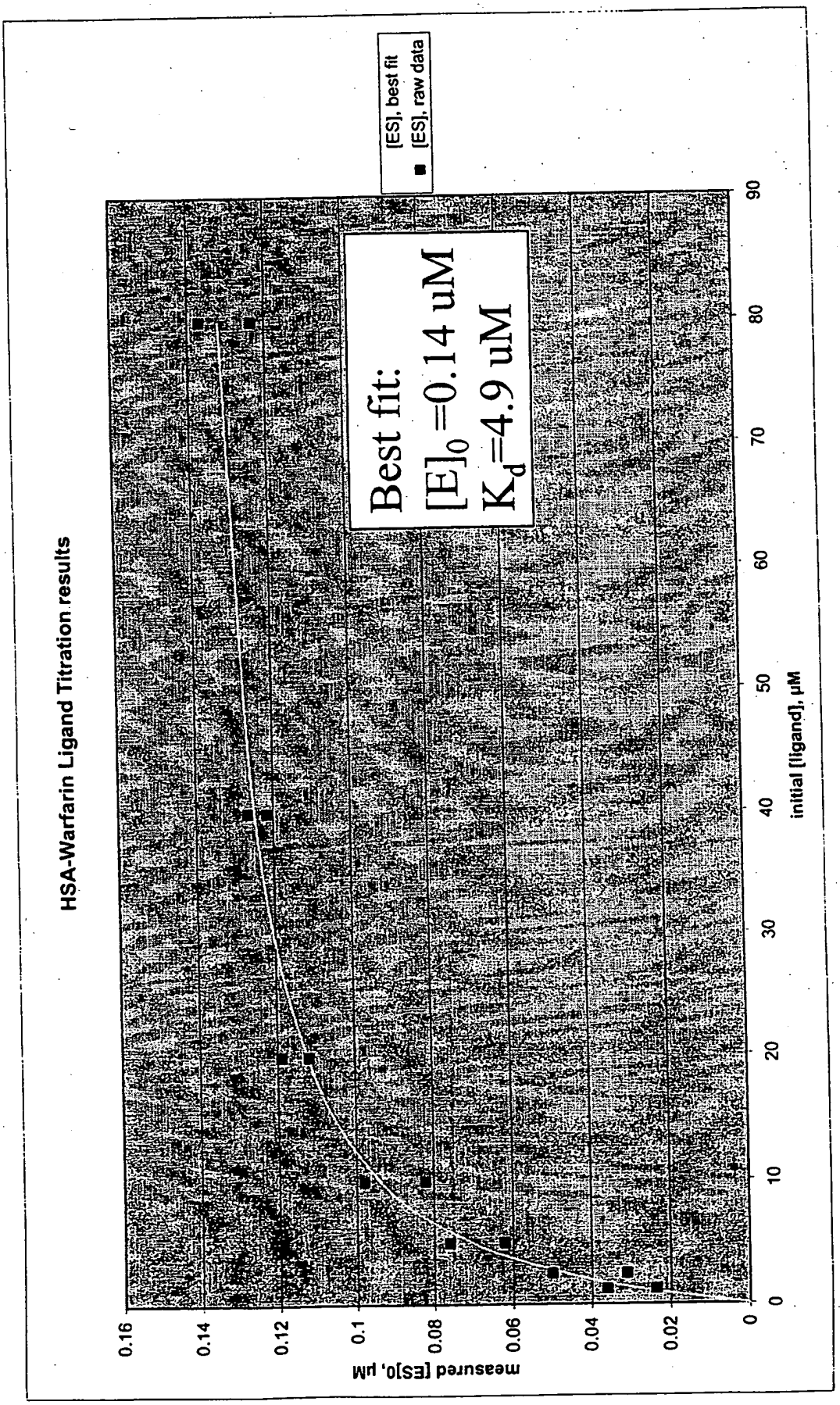
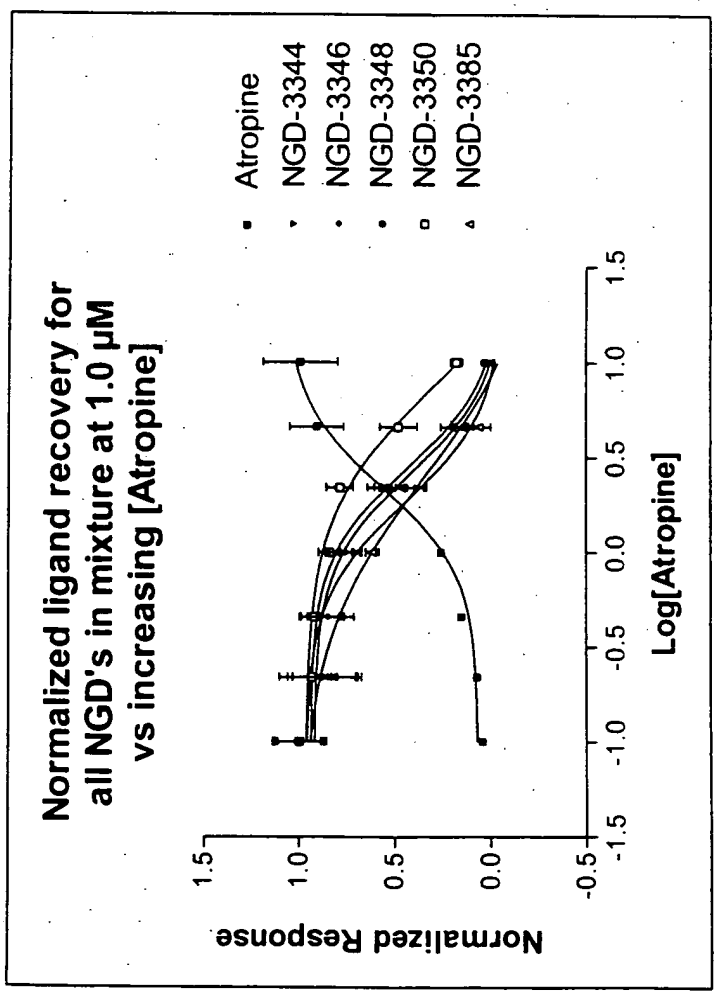
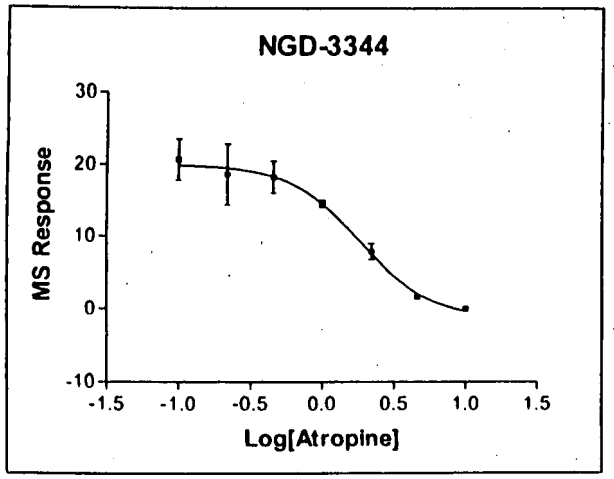

HSA-Warfarin Ligand Titration results
[ES], best fit
[ES], raw data
Best fit:
$[E]_0$=0.14 uM
K_d=4.9 uM
measured [ES]0, µM
initial [ligand], µM
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
40
50
60
70
80
90

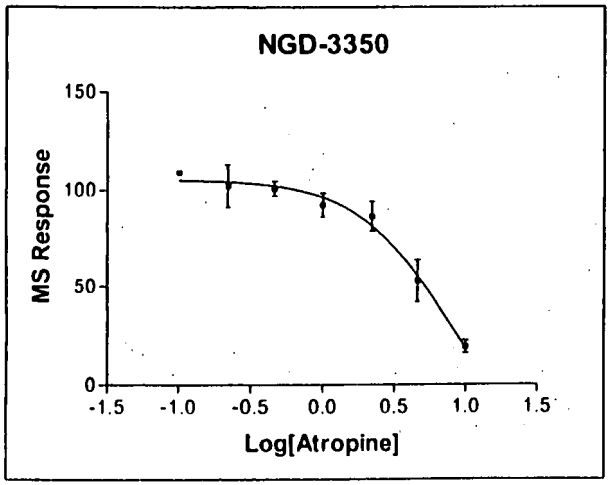
FIGURE 24



	ACE50, µM
Atropine	2.2
NGD-3344	1.8
NGD-3346	2.6
NGD-3348	2.7
NGD-3350	7.1
NGD-3385	2.3

FIGURE 25





- Comparison of NGD-3344 (weak) and NGD-3350 (strong) ligands shown
- K_d of ligands in mixture calculated from ACE_{50} given K_d of inhibitor (0.010 μM) & protein concentration = 2.0 μM

	ACE50, μM	Kd, μM
NGD-3344	1.8	0.75
NGD-3346	2.6	0.20
NGD-3348	2.7	0.19
NGD-3350	7.1	0.03
NGD-3385	2.3	0.30

FIGURE 26

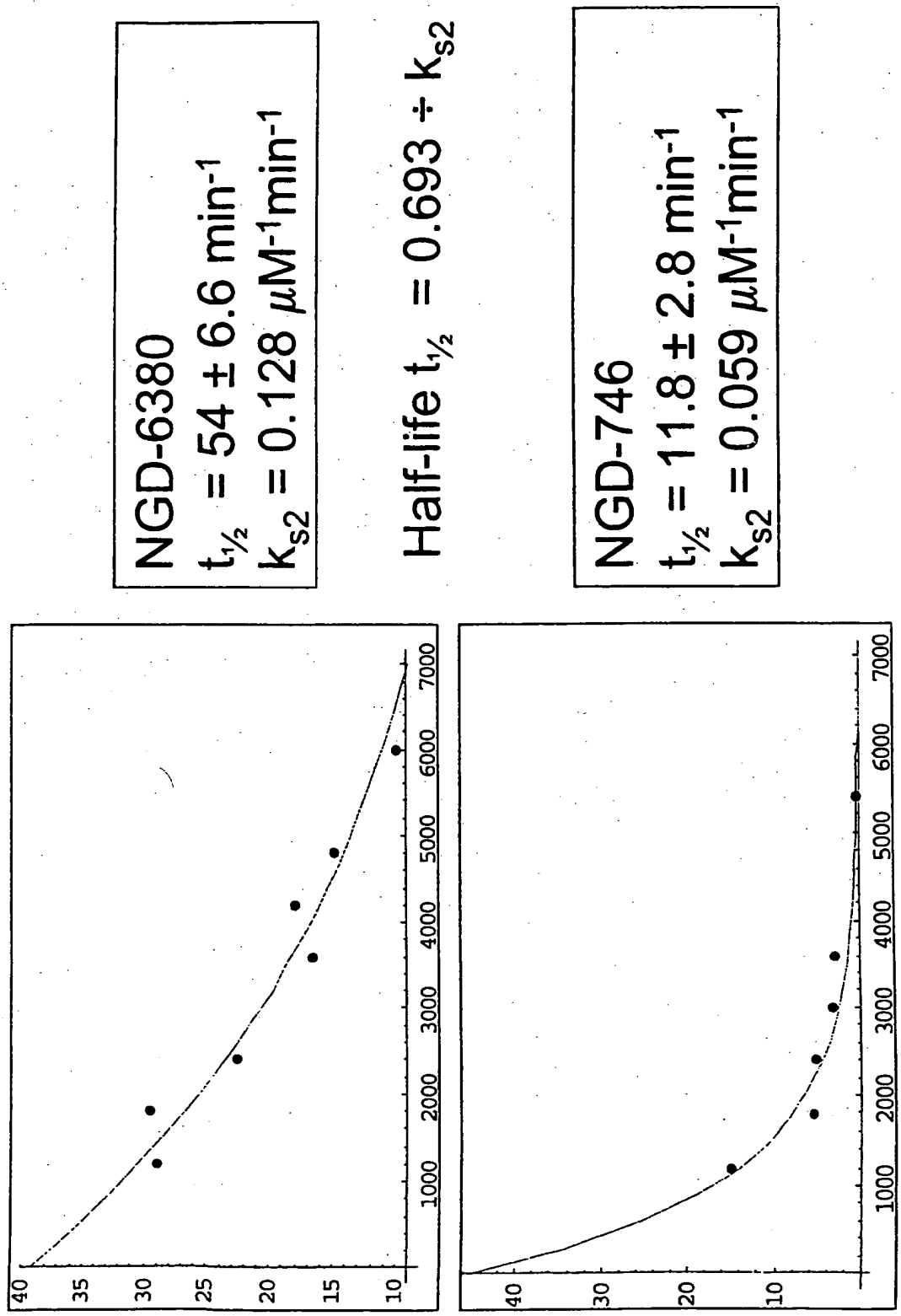
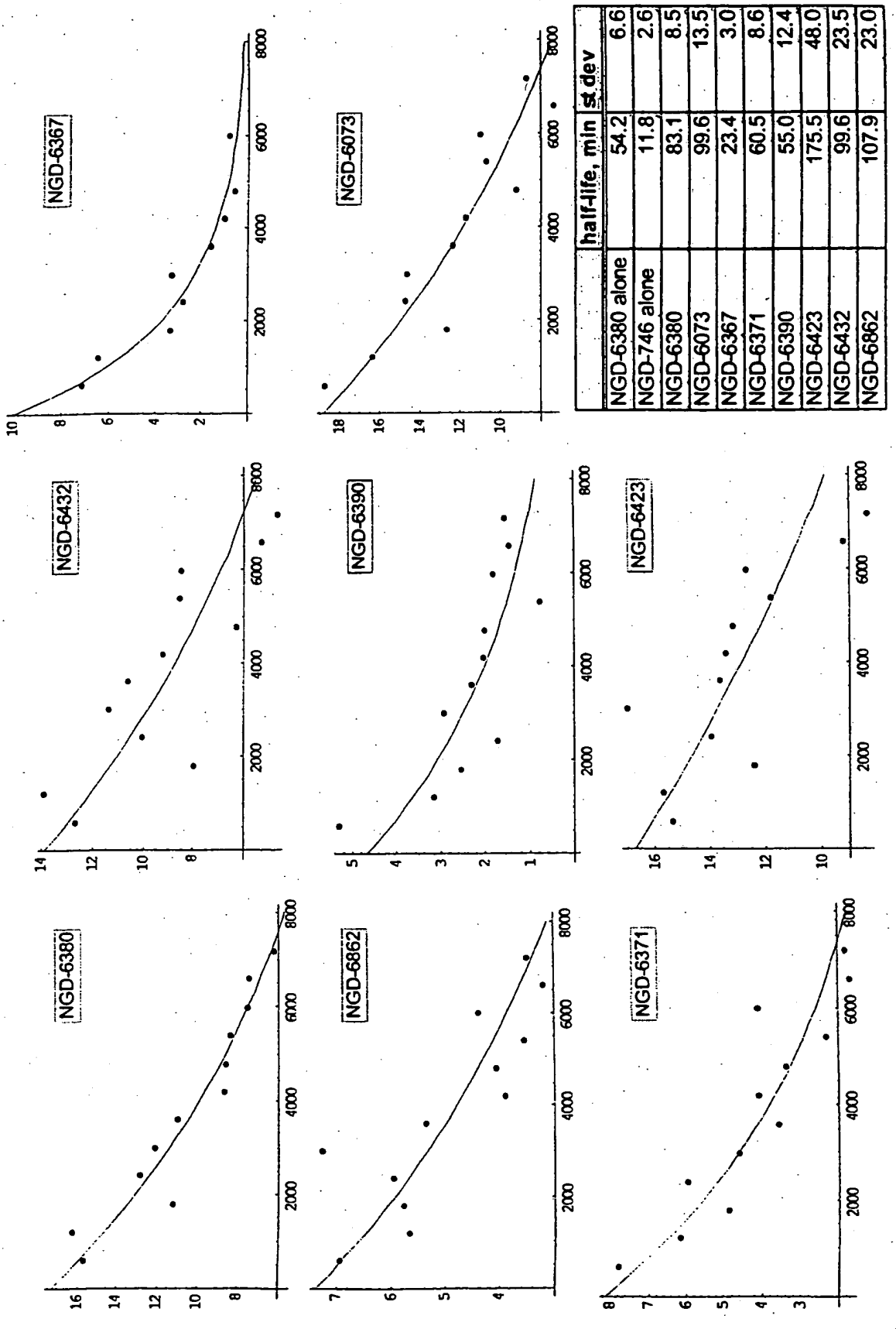
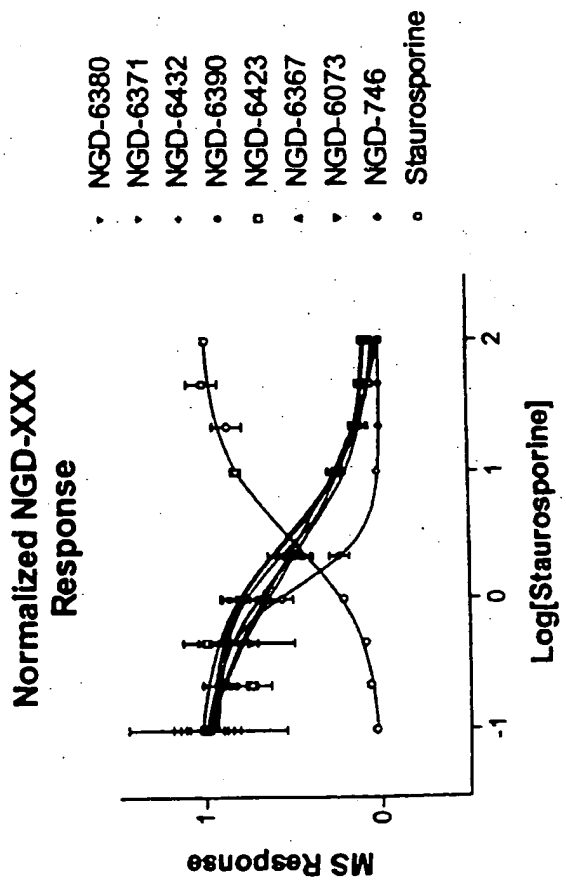

NGD-6380
$t_{1/2}$ = 54 ± 6.6 min^{-1}
k_{s2} = 0.128 μM^{-1}min^{-1}
Half-life $t_{1/2}$ = 0.693 ÷ k_{s2}
NGD-746
$t_{1/2}$ = 11.8 ± 2.8 min^{-1}
k_{s2} = 0.059 μM^{-1}min^{-1}
40
35
30
25
20
15
10
1000
2000
3000
4000
5000
6000
7000

FIGURE 27



	half-life, min	st dev
NGD-6380 alone	54.2	6.6
NGD-746 alone	11.8	2.6
NGD-6380	83.1	8.5
NGD-6073	99.6	13.5
NGD-6367	23.4	3.0
NGD-6371	60.5	8.6
NGD-6390	55.0	12.4
NGD-6423	175.5	48.0
NGD-6432	99.6	23.5
NGD-6862	107.9	23.0

FIGURE 28

	ACE50, µM	Kd, nM
NGD-6380	3.27	27
NGD-6862	n/d	
NGD-6371	3.03	30
NGD-6432	1.93	53
NGD-6390	3.04	29
NGD-6423	2.69	34
NGD-6367	2.44	39
NGD-6073	1.93	52
NGD-746	0.58	500